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Nop53p is required for late 60S ribosome subunit maturation and nuclear export in yeast

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ABSTRACT

We report that Ypl146cp/Nop53p is associated with pre-60S ribosomal complexes and localized to the nucleolus and nucleoplasm. In cells depleted of Nop53p synthesis of the rRNA components of the 60S ribosomal subunit is severely inhibited, with strikingly strong accumulation of the 7S pre-rRNA and a 5' extended form of the 25S rRNA. In cells depleted of Nop53p pre-60S subunits accumulate in the nucleus. However, a heterokaryon assay demonstrated that Nop53p is not transferred between nuclei, indicating that it is not released into the cytoplasm. We conclude that Nop53p is a late-acting factor in the nuclear maturation of 60S ribosomal subunits, which is required for normal acquisition of export competence. The strong accumulation of preribosomes in the Nop53p-depleted strain further suggests that it may participate in targeting aberrant preribosomes to surveillance and degradation pathways.

Keywords: nucleolus; ribosome synthesis; *Saccharomyces cerevisiae*; RNA surveillance

INTRODUCTION

The 80S yeast ribosome is composed of a large 60S subunit, containing the 25S, 5.8S, and 5S rRNA, and a small 40S subunit containing only the 18S rRNA species. The 18S, 5.8S, and 25S rRNA species are co-transcribed as a 35S polycistronic precursor in the nucleolus. The 35S pre-rRNA undergoes a series of endonucleolytic cleavages and exonucleolytic processing steps to yield the mature species (see Fig. 1). This process has been extensively studied in the genetically tractable model organism *Saccharomyces cerevisiae*, and most intermediate steps are well characterized.

Processing of the pre-rRNAs occurs within preribosomal particles that contain, in addition to pre-rRNA and ribosomal proteins, a plethora of transacting processing, modification and assembly factors, most of which are essential for cell viability. These nonribosomal proteins form transient interactions with the preribosomal particles, generating dynamic structures with continuously changing protein compositions. Affinity purification techniques (Rigaut et al. 1999) have allowed the composition of several 90S, pre-40S, and pre-60S complexes to be elucidated. The

earliest complex identified corresponds to a 90S particle, containing the 35S rRNA precursor in addition to factors implicated in 40S synthesis (Dragon et al. 2002; Grandi et al. 2002). Pre-rRNA cleavage at site A₂, within the 90S particle, gives rise to pre-60S and pre-40S particles. The pre-40S subunit is believed to then rapidly leave the nucleolus and traverse the nucleoplasm and nuclear pore complexes to the cytoplasm, where maturation is completed (Schafer et al. 2003). In contrast, the 60S subunit undergoes extensive rearrangement and processing within the nucleolus and nucleoplasm prior to its export to the cytoplasm. 60S maturation therefore takes longer than 40S synthesis, and more intermediate particles have been identified (Baßler et al. 2001; Harnpicharnchai et al. 2001; Saveanu et al. 2001; Fatica et al. 2002; Nissan et al. 2002; Saveanu et al. 2003). In both pathways, early particles are nucleolar, intermediate stage particles are nucleolar and nucleoplasmic, while late processing complexes are nucleoplasmic/cytoplasmic (for review, see Tschochner and Hurt 2003). The transit of the maturing preribosomes through the nucleolus, nucleoplasm, and cytoplasm, may provide an important means to spatially and temporally separate the steps in ribosome assembly and processing, and to ensure that they take place in the prescribed order.

Ypl146c/Nop53p was identified as a factor copurifying with components of the nuclear pore complex (Rout et al. 2000), as were many other late-acting ribosome synthesis factors. Nop53p was subsequently purified as a component

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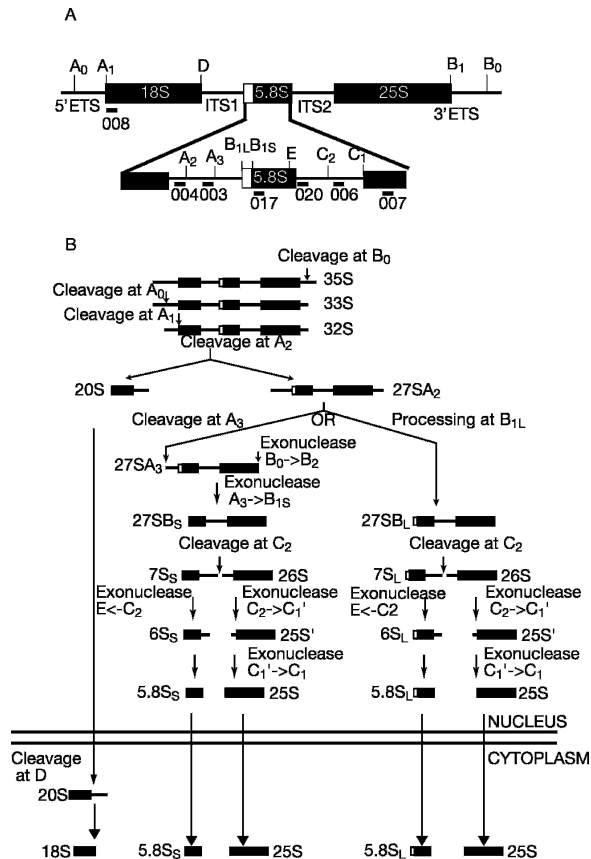


FIGURE 1. The pre-rRNA processing pathway in *Saccharomyces cerevisiae*. (A) The structure of the 35S rRNA precursor and locations of processing sites. The pre-rRNA encodes the 18S, 5.8S, and 25S rRNAs, which are flanked by the 5' and 3' external transcribed spacers (5'ETS and 3'ETS) and separated by internal transcribed spacers 1 and 2 (ITS1 and ITS2). (B) The pre-rRNA processing pathway. The 35S precursor is generated by co-transcriptional cleavage at site B₀ within the 3'ETS. This is followed by endonucleolytic cleavages at sites A₀ within the 5'ETS, A₁ at the 5' end of the mature 18S rRNA and A₂ within ITS1. Cleavage at A₂ separates the precursors to the 40S and 60S subunits and generates the 20S and 27SA₂ pre-rRNAs. 20S is subsequently exported from the nucleus to the cytoplasm where maturation to 18S is completed. The 27SA₂ pre-rRNA follows one of two alternate pathways: around 85% is cleaved at the A₃ site within ITS2, followed by 5'-3' exonucleolytic processing generating the 27SB₃ pre-rRNA. The remaining 15% is processed at site B_{1L}, which is located 8 nt 5' to B_{1S}, yielding the 27SB_{1L} pre-rRNA. These two alternate forms of 27SB are cleaved within ITS2 at site C₂, yielding 26S pre-rRNA and the long and short forms of 7S. The 7S pre-rRNAs are converted to mature 5.8S_L and 5.8S_S by a multistep 3' exonuclease pathway. Maturation of 26S to 25S rRNA similarly proceeds by a two-step 5' exonuclease pathway.

of a number of late pre-60S particles, including those defined by Cic1p, Nug1p, Ipi2p, Sda1p, Arx1p, and Nog1p (Baßler et al. 2001; Saveanu et al. 2001; Nissan et al. 2002). The early Cic1p-associated complex is localized to the nucleolar compartment, the Nug1p complex is enriched in both the nucleolus and the nucleoplasm, while the later complexes defined by Ipi2 and Sda1 are confined to the nucleoplasm. The most mature complex

containing Nop53p, the Arx1p-associated particle, localizes to the nucleoplasm and cytoplasm. In addition, Nop53p was found to associate with Trf4p (Ho et al. 2002), a protein that was recently implicated in pre-rRNA surveillance (LaCava et al. 2005; Wyers et al. 2005).

Here we describe the role of Ypl146cp/Nop53p in the processing of rRNA precursors and nuclear export of pre-60S ribosomal subunits.

RESULTS

Nop53p is a conserved protein of 52.55 kDa, the human homolog of which encodes GLTSCR2, a predicted glioma tumor suppressor (Smith et al. 2000). The protein has an unusual composition: Nop53p has 43% charged (DEHKR) residues (database average is 25%) and 39% hydrophobic (ACFGHILMTVWY) (database average is 57%). The protein is predicted to be largely α -helical, with a strong predicted coiled-coil domain between residues 326 and 360. It is likely to form homo- or heterodimers of the leucine-zipper type, since it has three leucine residues in this region with perfect heptad separation (M. Dlakic, pers. comm.). It seems quite unlikely that Nop53p has an enzymatic function, but it may form protein-protein interactions that are important for the structures of the preribosomes.

Nop53p associates with pre-60S particles

Affinity purification of preribosomal particles suggested that Nop53p might be a component of pre-60S particles. To confirm its association with preribosomal particles we used a C-terminal fusion between NOP53 and a TAP tag at the genomic locus (Ghaemmaghami et al. 2003), expression of which remained under the control of the endogenous promoter. Growth of the tagged strain was indistinguishable from the wild type (data not shown), indicating that the fusion protein is functional. Co-sedimentation of Nop53-TAP with preribosomal particles was tested by fractionation of a cell lysate on a 10%–50% sucrose gradient. Western blot analysis of gradient fractions (Fig. 2A) showed that Nop53-TAP sediments in two broad peaks. Northern hybridization (Fig. 2B,C) reveals that the lower peak of Nop53-TAP co-sediments with 27S pre-rRNA species (Fig. 2B), consistent with its association with pre-60S particles. The slower sedimenting Nop53-TAP fraction may correspond to the free protein plus smaller complexes of ribosome synthesis factors. Similar sized nonribosomal complexes have been reported for several other ribosome synthesis factors (Fatica et al. 2002; Dez et al. 2004; Horsey et al. 2004).

We also attempted to use Nop53-TAP to purify associated preribosomes, but were unable to detect any coprecipitation of RNAs, suggesting that the tag is not accessible.

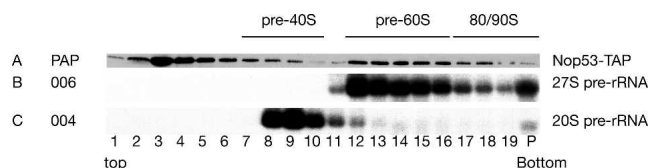


FIGURE 2. Nop53p co-sediments with pre-60S ribosomes. Cell lysate from a strain expressing Nop53-TAP was loaded on to a 10% to 50% sucrose gradient. (A) Western blotting using a peroxidase conjugated IgG (PAP) that recognizes Nop53-TAP. (B) Northern hybridization with a probe against the 27SA/27SB pre-rRNA components of pre-60S ribosomes. (C) Northern hybridization with a probe against the 20S pre-rRNA component of pre-40S ribosomes. The positions of sedimentation of pre-40S, 60S, and 90S particles are indicated. Fraction numbers are indicated, where 1 is the top gradient fraction and P is the pellet.

Nop53p is required for pre-rRNA processing

The *YPL146c/NOP53* ORF was initially reported to be essential in systematic deletion analyses (Winzeler et al. 1999). To characterize the role of Nop53p in ribosome synthesis we therefore constructed an N-terminal 3HA-nop53 fusion, under the control of the glucose repressible *GAL1* promoter, by one-step PCR (Longtine et al. 1998). Growth curves, RNA extractions, and Northern analyses were performed for two independently isolated *GAL::3HA-nop53* strains. Data are presented only for one strain, but very similar results were obtained from analyses of the second isolate (data not shown). On galactose containing complete medium the growth rates of the *GAL::3HA-nop53* strain and otherwise isogenic wild type were identical (data not shown). Following transfer to repressive, glucose medium, the growth rates of both strains were initially identical (Fig. 3A). However, growth of the *GAL::3HA-nop53* strain slowed progressively, commencing 3 h after transfer to glucose. Residual growth was observed for the *GAL::3HA-nop53* strain (12-h doubling time) even 24 h after transfer to glucose medium (data not shown). Western blot analysis (Fig. 3B) shows that the abundance of 3HA-Nop53p is strongly reduced after only 1 h on glucose media. It is possible that the N-terminal 3HA tag may destabilize the protein leading to its rapid turnover. Growth of the *GAL::3HA-nop53* strain was retarded on semipermissive, YPGRS medium (1% yeast extract, 2% bacto-peptone, 2% galactose, 2% raffinose, 2% sucrose) that reduces the level of expression. The apparently normal growth of the *GAL::3HA-nop53* strain on galactose may therefore be due to the overexpression that is often seen for GAL-regulated constructs during growth on galactose.

Ribosome synthesis in the Nop53p-depleted strain was assessed by pulse-chase labeling with [8-³H] adenine 1 h after glucose addition (Fig. 4). In the Nop53p-depleted strain, adenine incorporation into pre-rRNA was reduced approximately fourfold (estimated from the relative exposures

required to give approximately equal signals for the wild-type and Nop53p-depleted samples). Analysis of the high molecular weight RNAs (Fig. 4A) revealed that maturation of the 35S primary transcript and 32S pre-rRNA was mildly delayed, as shown by their persistence in later time points in the depleted strain and increased abundance relative to the 27S and 20S pre-rRNA compared to the wild type. Consistent with this, the appearance of the 27SA, 27SB, and 20S pre-rRNA also showed some delay. The most striking phenotype was the almost complete loss of synthesis of the mature 25S rRNA, whereas 18S synthesis continued. Analysis of low molecular weight RNAs (Fig. 4B) showed that the synthesis of the mature 5.8S rRNA was also greatly inhibited, whereas the 5S rRNA, which is independently transcribed, was much less affected. Notably, accumulation of the 7S pre-rRNA was similar in the Nop53p-depleted and wild-type cells. Relative to the wild type, the level of the 7S pre-rRNA is substantially higher than that of the 27SB pre-rRNA, its immediate precursors (see Fig. 1B). An experiment with longer pulse and chase times (Fig. 4C) showed that the accumulated 7S species persists over a long period, with very low levels of mature 5.8S synthesized.

Steady-state levels of mature rRNAs and pre-rRNAs were also analyzed by Northern hybridization and primer extension during depletion of 3HA-Nop53p. Total RNA was extracted during growth on galactose medium (0-h time points in Fig. 5) and at time points following transfer to

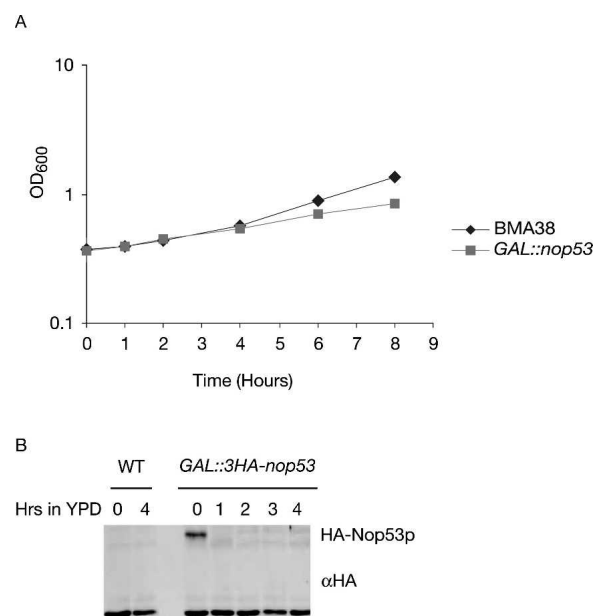


FIGURE 3. Depletion of 3HA-Nop53p rapidly inhibits cell growth. (A) Growth rate of wild-type (diamonds) and *GAL::3HA-nop53* (squares) strains following a transfer from permissive galactose medium to nonpermissive glucose medium for the times indicated. Cells were maintained in exponential growth throughout the time course by addition of prewarmed medium. (B) Western analysis of 3HA-Nop53p depletion. 3HA-Nop53p was decorated with rabbit anti-HA primary antibody, which was subsequently detected using antirabbit IgG linked to horseradish peroxidase.

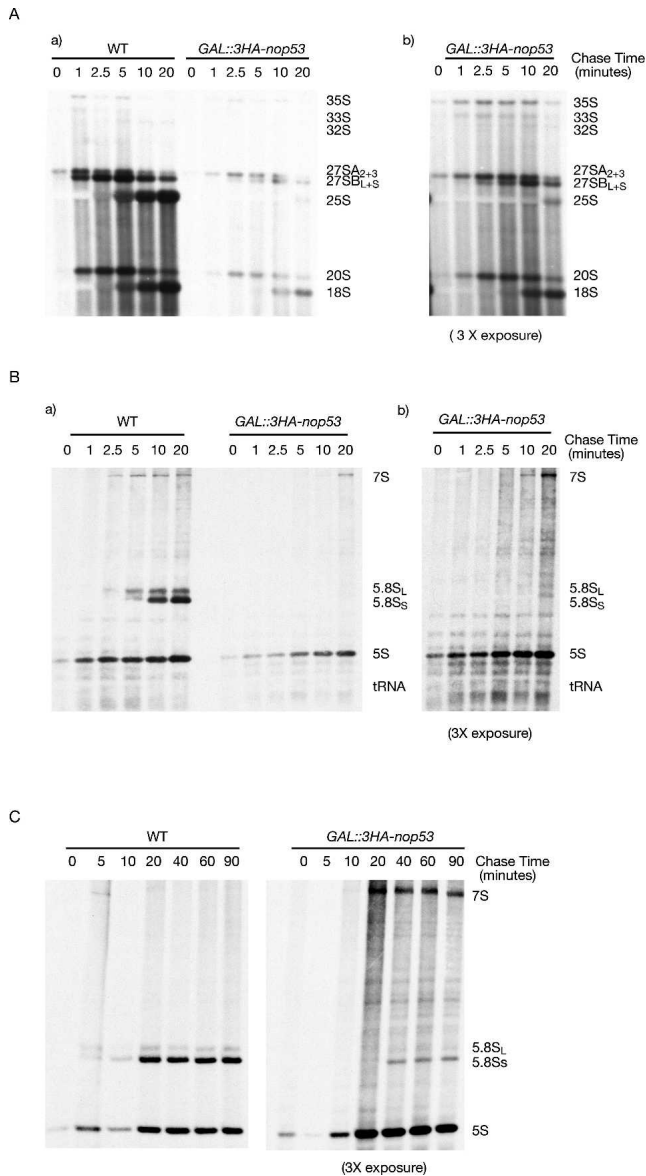


FIGURE 4. Depletion of Nop53p inhibits pre-rRNA processing. Wild-type and *GAL::3HA-nop53* strains were transferred to glucose containing medium for 1 h and then pulse labeled with [^3H] adenine for 2 min (A,B) or 5 min (C), followed by a chase with a large excess of cold adenine for the times indicated. (A) High molecular weight RNA was glyoxyl denatured and resolved on a 1.2% agarose gel. (B,C) Low molecular weight RNA was separated on 6% polyacrylamide/8.3 M Urea gels. In A and B, panel a shows the same exposure for the wild-type and *GAL::3HA-nop53* samples, while panel b shows a threefold longer exposure of the *GAL::3HA-nop53* samples. In C, the exposure shown for the *GAL::3HA-nop53* samples is threefold longer than for the WT.

glucose medium. Consistent with the pulse-chase data, depletion of Nop53p led to modest accumulation of the 35S and 32S pre-rRNAs (Fig. 5Aa). This was combined with the appearance of a low level of the aberrant 23S species (Fig. 5Ad), which is generated when cleavage at site A_3 precedes cleavage at sites A_0 , A_1 , and A_2 (see Fig. 1B). These early cleavage steps are essential for the

formation of the 20S pre-rRNA and the mature 18S rRNA component of the 40S ribosome. There was, however, little alteration in the levels of 20S pre-rRNA or 18S rRNA (Figs. 5Ad and e). A delay in these early cleavage steps is a common phenotype when factors required for 60S synthesis are depleted, probably due to indirect effects (for review, see Venema and Tollervey 1999). Mild increases were seen in the levels of the 27SA₂ and 27SB pre-rRNAs (Fig. 5Aa,b), but the 7S pre-rRNA was much more dramatically accumulated (Fig. 5Ba). PhosphorImager quantification 4 h after transfer to glucose medium, showed that the 7S pre-rRNA is 12-fold more abundant in the Nop53p depleted strain than in the wild type (data not shown).

Three forms of 3' extended 5.8S rRNA precursors exist in wild-type cells. The 7S pre-rRNA is extended to the C₂ cleavage site within ITS2, while the 5.8S+30 and 6S pre-

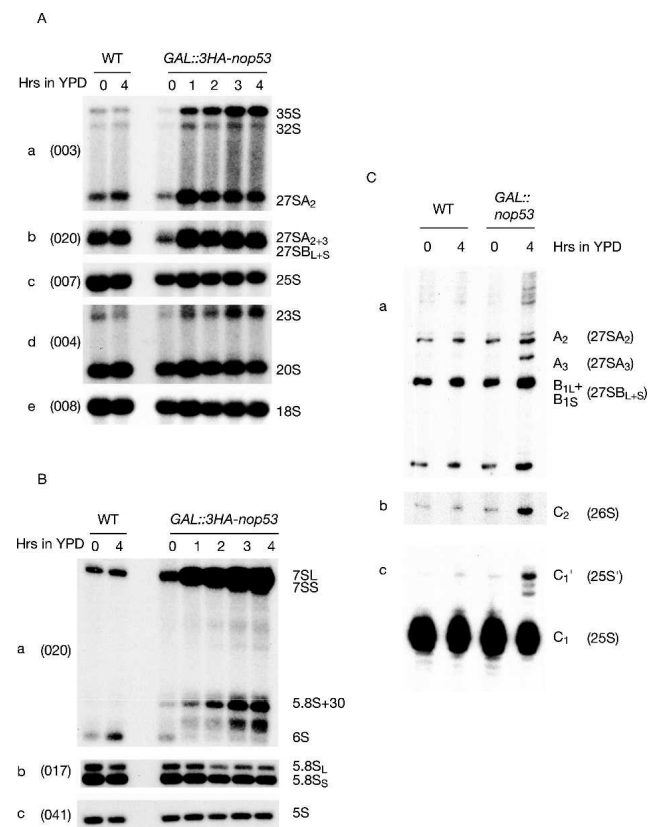


FIGURE 5. Nop53p is required for rRNA processing. Total RNA was extracted during growth on galactose medium (0-h time points) and at intervals following transfer to glucose media. The same RNA preparations were used in each analysis. (A) Northern analyses of high molecular weight RNA species resolved on a 1.2% agarose gel following glyoxyl denaturation. (B) Northern analyses of low molecular weight RNA species resolved on a 6% polyacrylamide/8.3 M urea gel; 4 μg of total RNA was used in Northern analyses and the hybridization probes are indicated on the left in parentheses. (C) Primer extension analysis utilizing an oligonucleotide that hybridized within the mature 25S rRNA, 40 nt from the 5' end (oligo 007). Pre-rRNA processing sites are indicated, with the corresponding pre-rRNA species indicated in parentheses. Each panel is taken from the same gel, but with different exposure times: (a) 16 h, (b) 40 h, and (c) 6 h.

rRNAs represent intermediates in the 3' maturation of 7S to mature 5.8S rRNA. The increase in 7S levels was combined with a modest accumulation of the 5.8S+30 pre-rRNA and the disappearance of the 6S precursor (Fig. 5Ba). This pattern indicates that 3' processing of the 7S pre-rRNA by the exosome complex is inhibited, as is subsequent processing of 5.8S+30, which specifically requires the Rrp6p component of the nuclear exosome (Briggs et al. 1998; Allmang et al. 1999). Short and long forms are detected for the mature 5.8S rRNA and its precursors, due to 5' end heterogeneity that results from the use of alternative processing pathways in ITS1 (see Fig. 1B). The ratios of long to short forms were unaltered during depletion of Nop53p (Fig. 5Bb). Little alteration was seen in the levels of the mature rRNAs during the time course analyzed, but these RNAs are stable and are depleted only slowly by growth.

Primer extension analyses (Fig. 5C) were performed using oligo 007, which hybridizes within the 25S region of the pre-rRNA (see Fig. 1A). Consistent with the Northern hybridization data, mild increases in the primer-extension stops at sites A₂ and B₁ were seen in the Nop53p-depleted strain (Fig. 5Ca), reflecting accumulation of the 27SA₂ and 27SB pre-rRNAs. The 27SA₃ pre-rRNA, which cannot be readily detected in Northern analyses, was accumulated in the Nop53p-depleted strain, as shown by the stop site at A₃ (Fig. 5Ca). Its level was, however, significantly lower than those of the 27SA₂ or 27SB pre-rRNAs, indicating that the delay in its maturation is modest. Pre-rRNA cleavage at site C₂ generates both the 7S and the 26S pre-rRNA species (Fig. 1B). A moderate accumulation of 26S pre-rRNA was seen following depletion of Nop53p, together with the accumulation of a shorter 5' extended 25S (25S') species identified by the C₁' stop. These observations show the inhibition of 5'-3' exonuclease processing during the final stages of 25S maturation. In Figure 5C the exposure shown for the C₂ stop (Fig. 5Cb) is 6.5-fold longer than for C₁' (Fig. 5Cc), indicating that the amount of accumulated 25S' pre-rRNA was substantially greater than 26S.

The 25S' pre-rRNA, which is 5' extended by ~6 nucleotides relative to the mature 25S rRNA, was previously shown to be associated with late, nucleoplasmic pre-60S complexes (Gadal et al. 2002; Saveanu et al. 2003). It seems likely that the accumulated 7S seen in Nop53p-depleted strains is associated with late pre-ribosomes that also contain the 25S' pre-rRNA.

Nop53p is a nuclear protein required for the export of the large subunit

To determine the localization of Nop53p, a construct expressing a C-terminal GFP fusion protein was integrated at the endogenous locus using a PCR strategy (Longtine et al. 1998). The nucleolus was identified in fixed cells by indirect immunofluorescence using a mouse anti-Nop1p antibody

(Wu et al. 1998) and a goat antimouse alexafluor-555 conjugated secondary antibody. The nucleoplasm was visualized with DAPI. Nop53-GFP localized to both the nucleolus and the nucleoplasm (Fig. 6A). Additionally a halo of GFP appeared around the DAPI and Nop1p signals, possibly corresponding to the nuclear envelope and/or nuclear pore complexes. It was previously reported that Nop53p interacts with components of the nuclear pore complex (Rout et al. 2000). The pattern of Nop53-GFP localization would be consistent with association with late pre-60S particles.

To ascertain whether Nop53p accompanies pre-60S particles from the nucleus through the NPC to the cytoplasm a heterokaryon assay was performed. A strain expressing Nop53-GFP was crossed with a *kar1-1* strain, in which mating and cell conjugation is not followed by

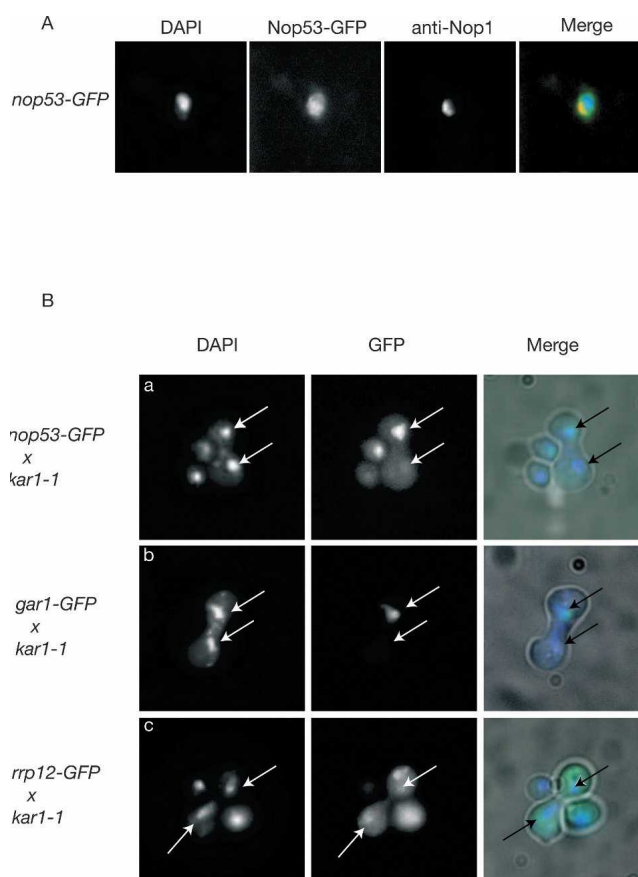


FIGURE 6. Nop53p is restricted to the nucleolus and nucleoplasm. (A) Nop53-GFP localizes to both the nucleolus and nucleoplasm. The nucleolus was visualized using an anti-Nop1p antibody (Wu et al. 1998), which was subsequently recognized by an Alexafluor conjugated secondary antibody. The nucleoplasm was visualized by DAPI staining. (B) Nop53p does not shuttle from the nucleus to the cytoplasm. Strains expressing Nop53-GFP, Gar1-GFP, and Rrp12-GFP were grown in YPD media, mated with a *kar1-1* mutant strain, and incubated at 25°C until heterokaryons formed. Localization of the GFP-tagged proteins in heterokaryotic cells is shown for (a) Nop53-GFP, (b) Gar1-GFP, and (c) Rrp12-GFP. Cells are shown with DAPI stained nucleoplasm. GFP and DAPI signals are also shown merged with DIC bright field images. Arrows indicate the positions of nuclei in the heterokaryons.

nuclear fusion, leading to heterokaryon formation (Vallen et al. 1992). Nop53-GFP was localized to only one of the nuclei in the heterokaryons (Fig. 6Ba), indicating that the protein does not actively shuttle between the nucleus and the cytoplasm. The characterized nuclear-cytoplasmic shuttling protein Rpl12-GFP was found in both nuclei of the heterokaryon (Fig. 6Bc), while the nonshuttling nucleolar protein Gar1-GFP was present in only one of the nuclei in heterokaryons (Fig. 6Bb; Oeffinger et al. 2004). We conclude that Nop53p dissociates from pre-60S particles prior to or during the process of export to the cytoplasm.

To determine whether Nop53p is required for nuclear export of the large ribosomal subunit, the localization of the 60S reporter construct Rpl11b-eGFP (Stage-Zimmermann et al. 2000) was analyzed in wild-type and *GAL::3HA-nop53* strains. Under permissive conditions Rpl11b-eGFP was found throughout the cell in both wild-type and mutant cells (Fig. 7Aa,c). Following a shift to nonpermissive glucose media for 2 h, nuclear accumulation of the Rpl11b-eGFP signal was visible in the *GAL::3HA-nop53* strain (Fig. 7Ad), and by 4 h the accumulation was substantial (Fig. 7Ae).

As shown in Figure 7B, nuclear accumulation of Rpl11p-GFP was observed in both the nucleoplasm (shown by the DAPI-stained region) and in the nucleolus (decorated by antibodies against the nucleolar marker Nop1p). No accumulation of 40S particles was seen in the *GAL::3HA-nop53* strain as judged by the localization of Rps2p-eGFP (data not shown) (Milkereit et al. 2003).

DISCUSSION

The data presented here establish that Ypl146cp/Nop53p is a late-acting nuclear component of the 60S ribosome synthesis machinery. Sucrose gradient analyses showed the co-sedimentation of Nop53-TAP with the 27S pre-rRNA component of the pre-60S particles. Depletion of Nop53p under the control of a repressible *GAL* promoter severely inhibited the synthesis of the 25S and 5.8S rRNA components of the mature 60S subunits. In the absence of Nop53p pre-60S particles accumulate in the nucleus, as judged by the nuclear retention of the Rpl11b-eGFP reporter. These pre-60S particles are likely to also contain the 7S and 25S' pre-rRNAs, which were strongly accumulated in Nop53p-depleted strains. This indicates that the presence of Nop53p is required for pre-60S particles to attain export competence. Depletion or mutation of several different proteins inhibits both 60S subunit export and processing of 7S pre-rRNA to 5.8S (Gadal et al. 2001a, 2002; Nissan et al. 2002; Galani et al. 2004; Oeffinger et al. 2004), although no previously characterized mutation resulted in the very high levels of 7S accumulation observed following depletion of Nop53p. Maturation of 7S pre-rRNA to 5.8S rRNA is reported to occur in the nucleoplasm (Nissan et al. 2002), indicating that it may be directly linked to the acquisition of export

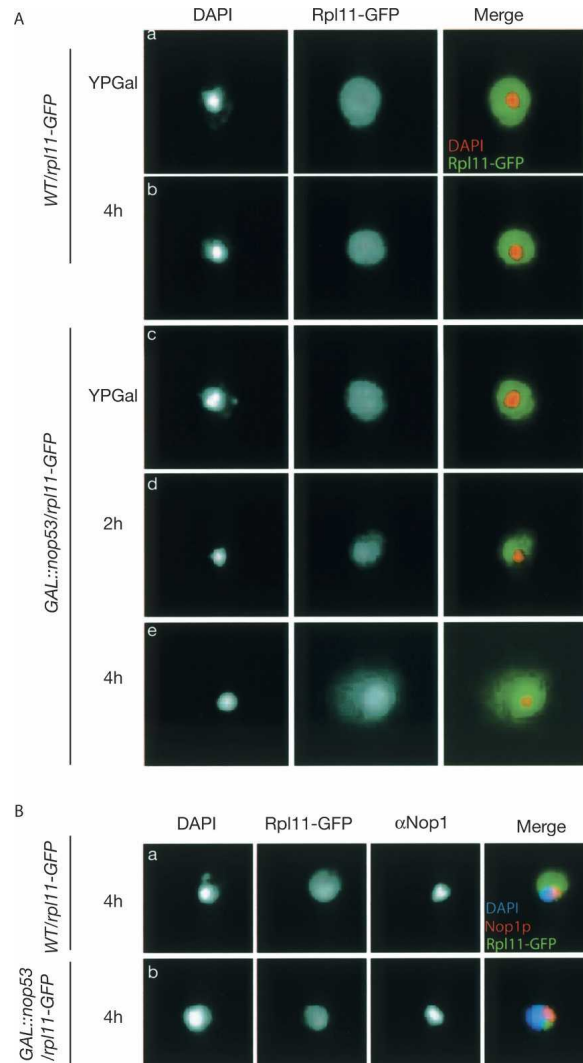


FIGURE 7. Nop53p is required for the nuclear-cytoplasmic export of the 60S subunit. (A) Wild-type and *GAL::3HA-nop53* strains were transformed with a centromeric plasmid expressing Rpl11b-eGFP (shown in green). Cells were pregrown in galactose synthetic medium lacking leucine (a,c) and shifted to YPD for the times indicated (b,d,e). Cells were fixed and stained with DAPI to visualize the nucleoplasm (shown in red). (B) Wild-type (a) and *GAL::3HA-nop53* strains (b) containing pRpl11b-GFP (shown in green) were shifted to YPD for 4 h. The nucleolus was visualized using an anti-Nop1p antibody (shown in red), and nucleoplasm was stained with DAPI (shown in blue).

competence, or to the export process itself. Studies in *Xenopus* oocytes (Trotta et al. 2003) have also linked 3' maturation of the 5.8S rRNA to 60S subunit export, suggesting that this is a conserved feature.

While Nop53p is implicated in the nuclear export of pre-60S subunits, a heterokaryon assay demonstrated that Nop53-GFP does not transfer between nuclei. This indicates that Nop53p does not accompany pre-60S particles into the cytoplasm. We hypothesize that Nop53p mediates an interaction between the pre-60S particles and the

nuclear pore complex, consistent with the reported association of Nop53p with nucleoporins (Rout et al. 2000). Nop53p may dissociate prior to subunit translocation through the lumen of the pore, or be rapidly released and reimported following emergence of the preribosome on the cytoplasmic face of the NPC.

It seems likely that an important quality control system monitors subunit integrity prior to export to the cytoplasm. Only pre-60S subunits that are accurately assembled and sufficiently mature gain export competence. Export to the cytoplasm is presumably an irreversible step that takes the subunits beyond the reach of the nuclear RNA surveillance system, which appears to be more active than cytoplasmic surveillance. When the first mutants defective in subunit export were identified, it was assumed that the corresponding proteins functioned as components of the ribosomal subunit export machinery (Stage-Zimmermann et al. 2000). However, as the numbers of such “export factors” has grown, it has become increasingly unlikely that they all function directly in subunit export (Stage-Zimmermann et al. 2000; Bähler et al. 2001; Gadai et al. 2001a,b; Gleizes et al. 2001; Milkereit et al. 2001; Oeffinger et al. 2002, 2004; Fatica et al. 2003; Kallstrom et al. 2003; Milkereit et al. 2003; Galani et al. 2004). Rather, many mutations may inhibit export because they generate preribosomes that are perceived to be defective by a surveillance system that monitors the export-competence of the subunits.

NOP53 was initially reported to be essential for viability, based on a high-throughput screen. However, a very recent analysis found that a *nop53Δ* deletion strain is viable, although impaired in growth (Sydorsky et al. 2005). The large majority of characterized yeast ribosome synthesis factors are essential for viability, and conditional mutants fail to synthesize one or more rRNA species under non-permissive conditions. In most cases, they also accumulate only low levels of pre-rRNA, indicating that surveillance of preribosomes and degradation of pre-rRNAs are very active in yeast. This is in marked contrast to the situation in *Escherichia coli*, where ribosome synthesis is not known to be subject to surveillance activities. Mutations in ribosome synthesis factors are not lethal in *E. coli*, and generally lead to the accumulation of preribosomes and the synthesis of at least partially functional ribosomes (for review, see El Hage and Tollervey 2004). In this context, Nop53p is unusual among yeast ribosome synthesis and export factors, since its depletion resulted in a dramatic accumulation of the 7S pre-rRNA. This indicates that the aberrant pre-60S ribosomes accumulated in this background are not subject to rapid degradation. Moreover, since Nop53p is nonessential, functional ribosomes must be synthesized in its absence. These observations would be consistent with the model that the lethality of many ribosome synthesis factors is a consequence of active surveillance.

The exosome complex of 3'-5' exonucleases is known to degrade aberrant pre-rRNAs that arise from the inhibition

of processing (Allmang et al. 2000), and is a likely candidate to also degrade nuclear-restricted preribosomes. The exosome can be activated for RNA degradation in vitro and in vivo by the nuclear “TRAMP” complex (Trf4p, Air, Mtr4p polyadenylation complex) (LaCava et al. 2005; Vanacova et al. 2005; Wyers et al. 2005). This is comprised of a poly(A) polymerase Trf4p, a zinc-knuckle protein—either Air1p or Air2p, which are functionally redundant—and the putative RNA helicase Mtr4p. Strains lacking components of the TRAMP complex accumulate the aberrant 23S pre-rRNA, which is a characterized substrate for the nuclear exosome (Allmang et al. 2000; LaCava et al. 2005). This indicates that the TRAMP complex functions together with the exosome in the surveillance and degradation of defective preribosomes. Nop53p was previously identified as protein that coprecipitated with FLAG-tagged Trf4p (Ho et al. 2002), suggesting the possibility that Nop53p also participates in the recognition of aberrant preribosomes. This would offer a potential explanation for the observation that preribosomes lacking Nop53p appear to be unusually resistant to RNA surveillance and degradation.

MATERIALS AND METHODS

Strains and microbiological techniques

Standard procedures were used for the propagation and maintenance of yeast. A full list of strains used in this study can be found in Table 1. YET1 and YET2 were constructed using a one-step PCR strategy (Longtine et al. 1998), during which transformants were selected for resistance to G418 and HIS prototrophy, respectively, and screened by PCR and immunoblotting. GAL conditional mutant and BMA38 wild type were transformed with pRpl11b-eGFP (kindly provided by P. Silver) to analyze the nuclear export of 60S subunit, and a pAde vector for pulse chase analysis.

For depletion of GAL regulated Nop53p, cells were pregrown on permissive media containing 2% galactose harvested at various time intervals following a shift to nonpermissive media containing 2% glucose. Strains containing pRpl11b-eGFP were pregrown in galactose minimum media lacking leucine.

Pulse chase analysis

Metabolic labeling of pre-rRNA was performed as previously described (Tollervey et al. 1993) with the following modifications. The strains *BMA38* and *GAL::3HA-nop53* were transformed with a plasmid containing *ADE2* gene. Strains were pregrown in synthetic galactose medium lacking adenine, and transferred to synthetic glucose medium lacking adenine for 1 h. Cells with an OD₆₀₀ 0.4 were labeled with 8-³H adenine for 2 or 5 min, followed by a chase of excess cold adenine. One-milliliter samples were spun down and cell pellets were frozen in liquid nitrogen. RNA was extracted and ethanol precipitated.

TABLE 1. Yeast strains used and constructed in this study

Strain	Genotype	Reference
BMA38	<i>MATa his3Δ200, leu2-3, 112, ura3-1, trp1Δ, ade2-1</i>	Baudin et al. 1993
YET1	As BMA38 but <i>KAN::GAL1::3HA-yp146c</i>	This study
BY4741	<i>MATa his3Δ1, leu2Δ0, met15Δ0, ura3Δ0</i>	Brachmann et al. 1998
Ypl146c-TAP	As BY4741 but <i>yp146c-TAP::HIS</i>	Ghaemmhami et al. 2003
YET2	As BMA38 but <i>yp146c-eGFP::HIS</i>	This study
YET3	As BMA38 but <i>pRpl11b-eGFP::LEU</i>	This study
YET4	As YET1 but <i>pRS315Rpl11b-GFP::LEU</i>	This study
YET5	As BMA38 but <i>pGar1-eGFP</i>	This study
MS740	<i>MATα ade2-101, ura3-52, leu2-3, leu2-112, kar1-1</i>	Vallen et al. 1992
YMO17	<i>MATa; ura3-52; trp1; leu2Δ1; his3Δ200; gal2; galΔ108Rpl12-eGFP::KAN</i>	Oeffinger et al. 2004
YET6	As BMA38 but <i>pAde</i>	This study
YET7	As YET1 but <i>pAde</i>	This study

RNA extraction, Northern hybridization, and primer extension

RNA was extracted as previously described (Tollervey and Mattaj 1987). For high molecular weight RNA analysis 4 μg of total RNA was glyoxyl denatured and resolved on a standard 1.2% agarose gel, as previously described (Sambrook and Russell 2001). Low molecular weight RNAs and primer extension products were resolved on standard 6% Acrylamide/8.3 M urea gels. Primer extension reactions were carried out on 4 μg of total RNA as previously described (Beltrame and Tollervey 1992).

Oligonucleotides for Northern hybridizations and primer extension:

- 003: 5'-TGCTTACCTCTGGGCC;
- 004: 5'-CGGTTTTAATTGTCCTA;
- 006: 5'-AGATTAGCCGCAGTTGG;
- 007: 5'-CTCCGCTTATTGATATGC;
- 008: 5'-CATGGCTTAATCTTTGAGAC
- 017: 5'-GCGTTGTTCATCGATGC;
- 020: 5'-TGAGAAGGAAATGACGCT;
- 041: 5'-CTACTCGGTCAGGCTC.

Sucrose gradient analysis

Sucrose gradient analysis was performed on a 10% to 50% gradient as previously described (Tollervey et al. 1993). RNA and protein was extracted from each fraction. RNA was resolved on standard 1.2% Agarose-formaldehyde gels and 6% acrylamide gels. Aliquots of each fraction were TCA precipitated and resolved using standard SDS-PAGE techniques. The sedimentation profile of Nop53-TAP was detected by immunoblotting with a peroxidase-conjugated rabbit IgG (Sigma).

Fluorescence and immunofluorescence microscopy

For immunofluorescence cells were fixed in 3.7% paraformaldehyde at room temperature and spheroplasted using zymolase. Nop1p was detected with a mouse anti-Nop1 antibody (kindly provided by J. Aris, University of Florida), and a secondary goat antimouse antibody conjugated to Alexafluor 555 (Molecular Probes). To stain nuclear DNA, DAPI was included in the

mounting medium (Vectashield, Vector laboratories) For Rpl11b-eGFP localization assays cells were pregrown in galactose synthetic media lacking leucine. Cells were shifted to YPD media and samples collected and fixed between 0 and 4 h at 30-min intervals.

Heterokaryon assay

Heterokaryon assays were carried out as previously described (Peng and Hopper 2000), with the following modifications. Mating was initiated by concentrating an equal number of Nop53-GFP cells and *kar1-1* (provided by M. Rose, Princeton University) cells on a 25-mm nitrocellulose filter with 0.45-μm pore size, and incubated at 25°C on a YPD plate. Cells were removed from the membrane and fixed following 1–3 h of incubation, at 30-min intervals. Microscopy was carried out on a Leica DMR fluorescence microscope and pictures were captured using a Coolsnap CCD camera.

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